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SUBJECT: FINAL REPORT

Project: *The Coupling of Solution Chemistry to Plant Nutrient Demand In An On Demand Nutrient Delivery System*, 21-1501-0984.

This project has been completed and results of the project are herewith forwarded to your office. This report will be the form the basis of a publication and a future grant proposal to NASA.

Plant stress can be the result of many different factors, each of which must be defined individually and can result in many different types of stress. Our ability to quantify stress is determined by our ability to quantify a reduction in some important attribute of the plant and to identify the causal agent of that reduction. Environmental stresses (extreme conditions of toxicity and deficiency) are well known to induce suppressed performance in plants. Several techniques can be utilized to identify plant stress depending on the type of stress. In the case of nutrient stress, a typical method of direct recognition is visual observation of foliar symptoms or direct measurement of plant tissue nutrient content. In general, severe suppression of plant performance has already occurred by the time visual symptoms of nutrient deficiency can be observed and direct measurement of tissue nutrient content requires time for processing and determination of nutrient content. Monitoring of gas exchange and determination of net photosynthesis is a powerful tool to measure plant performance that provides an integrated response of all growth factors. Gas exchange can provide a good measure of stress response provided a single stress can be identified as responsible. Gas exchange does not allow assignment of crop suppression from data collected from crops of multiple age or from multiple types of crops nor does it identify suppression resulting from multiple sources of stress.

The stages of plant growth and nutrient stress determined and quantified through measurement of net photosynthesis can be observed in Figure 1. Data in Figure 1 were recorded in our laboratory utilizing the model system and equipment planned to support this project. In this instance, a crop of wheat was grown in a closed, controlled-environment nutrient chamber where nutrient concentrations in solution, plant nutrient uptake, and net photosynthesis were tracked in addition to the standard environmental controls of the growth chamber.

Short-term Interactions of Nutrient Uptake and Photosynthesis

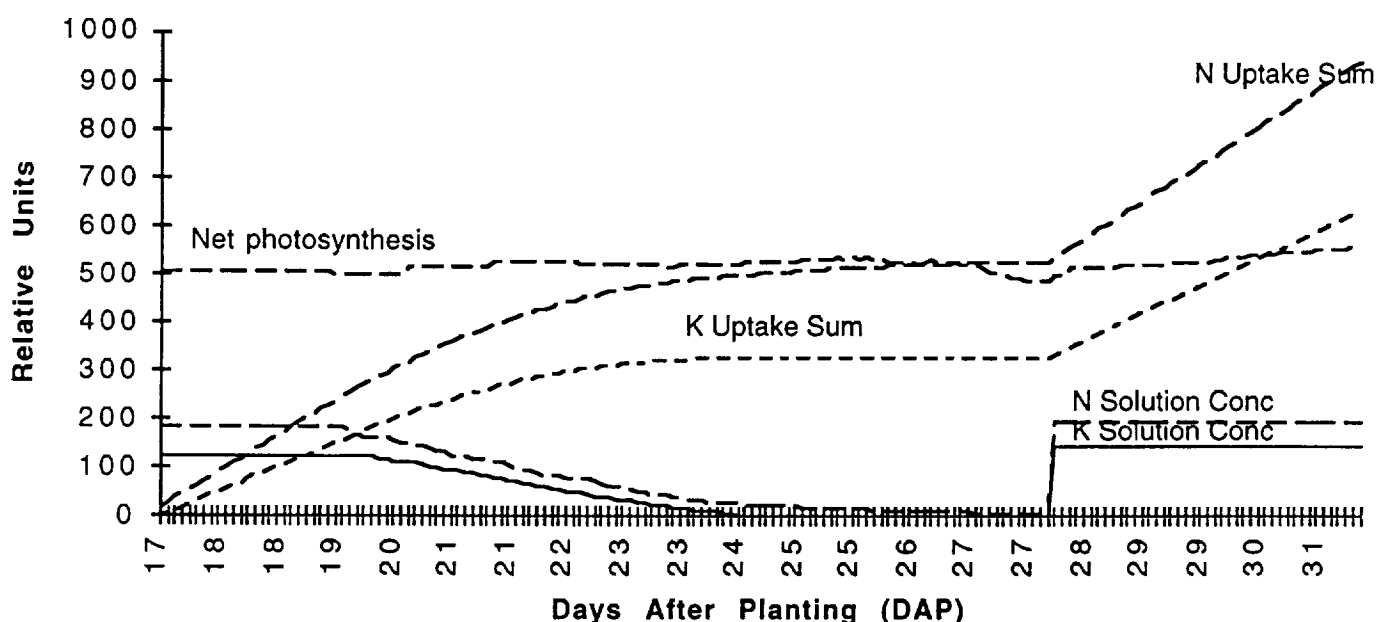


Figure 1

The wheat had reached canopy closure by 16 days-after-planting (DAP) establishing a reference net photosynthetic level. Beginning at 19 DAP, further additions of potassium (K) and nitrogen (N) were withheld. Between 19 and 24 DAP, the plants continued to grow at the reference rate of growth and take up nutrients as indicated by the net photosynthesis and the K and N uptake curves. At 24 DAP, the point when all potassium from the solution had been depleted by the plants, nutrient uptake sharply decreased for all other nutrients monitored. However, there was a three day lag between the time there was no longer a source of external K and the effect on net photosynthesis could be observed. This lag is likely the result of some degree of internal buffering and retranslocation of nutrients to the photosynthetic site. It is important to note that no visual symptoms of nutrient

deficiency were ever observed. At 27 DAP, nutrient injection to the solution was reinstated. Nutrient uptake occurred rapidly for approximately 18 hours following nutrient addition, at which time nutrient uptake decreased, to reestablish a near normal nutrient uptake pattern. The recovery of net photosynthesis was also rapid but exhibited a somewhat longer recovery time (almost 48 hours).

It is important to point out that no visual, foliar symptoms of nutrient deficiency were observed in plants from the study described in Figure 1. However, a strong suppression in net photosynthesis (CO_2 uptake, O_2 production, and plant growth) was observed to result from the nutrient stress whereas recovery of growth followed injection of nutrients at 27 DAP.

The goal for advanced life support is to provide self-sufficiency for human beings to carry out research and exploration productively in space. The objective for plant-based, regenerative life support is to optimize food loop closure, with concomitant air and water revitalization. To realize this objective in an operational life support system, methods must be in place to assess plant performance and manage crop production systems. Presently, gas exchange measurement (primarily photosynthesis) provides the best estimate of plant performance. Management of the system is accomplished by supplying inputs to the crop production system as determined by that required to maintain set points for measured parameters of the atmosphere (CO_2 , H_2O , temperature, etc.) and root zone nutrient solution (electrical conductivity and elemental concentration).

Gas exchange measurements can provide a good estimate of plant growth in a single crop system. This technique will, however, be difficult to implement in a mixed crop production system that is human tended. Monitoring the gas changes from the entrance of a closed crop chamber to its exit from that chamber and relating those changes to the function of the crop contained in the closed chamber is a reliable experimental method, but when integrated with humans and mixed crops it will provide little information regarding the growth of individual plant types or age class of a particular crop in the life support system. The presence of humans in the production area will make interpretation of the gas exchange information difficult because of the CO_2 they generate. Even if humans were isolated from the system by wearing breathing devices, gas exchange methods would still provide only an integrated performance estimate for the entire system; in order to achieve individual crop assessment, it would be necessary to require that each individual crop segment be independently sealed and the input and outlet gas qualities be monitored.

Deficiencies in gas exchange methods applied to assess plant performance in mixed crop production will also affect the ability to identify plant stress experienced by individual crops within the system. Any sensitivity of an individual crop to stress, such as pathogens or nutrient imbalance, will be difficult to assess other than through observation of visual symptoms. Presently, the control of nutrient delivery to most hydroponic crop production systems is based on monitoring and maintaining the desired elemental concentrations in the nutrient solution. Direct monitoring of the plant's nutrient level is not used as the primary control signal because of the difficulty of determining it in real time; thus, indirect

sensors are used for the maintenance of solution concentrations. It is well established that the nutrient requirement differs among various crops and for various developmental stages of a single crop. Long-term operation of a single or small number of hydroponic system supporting a mix of crop types with nutrient and water additions maintained on the current basis of solution electrical conductivity is prone to potential nutrient imbalance. The delivery of nutrients to crop production will become even more complex as the input of reagent grade nutrient salts used in research is replaced with resources recovered from waste processing. The precise quality of those recovered resources will depend on the specific technology used, but the prime candidates currently being included in regenerative life support (RLS), bioprocessors, and incineration, do not produce separate streams of high grade salts or even consistent concentration of the required nutrients.

Presently, the only functional methods available for monitoring plant nutritional status and biochemical quality is destructive sampling of plant material followed by laboratory analysis. The current state-of-the-art in monitoring plant health involves a combination of visual examination for observation of symptoms associated with nutritional deficiencies or toxicity and tissue and soil analysis to evaluate availability of nutrients. The observation of visual symptoms of plant stress provides an indication that a stress is present and has been present for an extended period of time. By the time that visual symptoms are observed the impact on plant-based life support function will have already been significant. Tissue analysis is destructive, requires time for processing of samples, and requires interpretation of sample data from the tissues sampled to whole plant and further to mixed crop performance.

Plant-based, regenerative life support systems are necessary to support likely future space exploration missions and Lunar/planetary outposts. The recycling of mass and regeneration of food, air, and water consumables is important for both the long-term safety of a crew and limiting the expense of re-supply logistics. Utilizing a plant-based life-support system to supply food, air and water to a crew requires that the system be reliable, stable and predictable. A proposal will be developed to unite imaging interferometry technology, originally developed in the remote sensing scientific community to assess the health and status of native plants, to address specific needs in the use of crop plants in regenerative life support. The specific technology is the Digital Array Scanned Interferometer (DASI) instrument.

The DASI addresses several important issues related to life support. It provides the potential for an assessment of environmental stress, nutritional status, water relations, and characterizes the biochemical quality of several important constituents of plant biomass, all important in maintaining transpiration, photosynthesis and food production. All of these are important in the assessment of plant performance in a regenerative life support application. Equally important is the potential of physiological data from regenerative life-support systems to provide calibration data for the DASI system.

The current gas exchange physiological methods utilized to monitor critical life support related plant performance (H_2O production, CO_2 removal, O_2 production, and biomass estimation) will likely be difficult to implement and certainly difficult to interpret in a human-tended, mixed-crop production application. Monitoring the gas changes from the entrance into a closed crop chamber and its exit from that chamber is a reliable experimental method, but when humans are integrated into the system, little information regarding the growth of individual plant types or age class of a particular crop will be available. The DASI system can produce a digital image of the entire cropping area allowing analysis of specific areas (pixels) within the image to assess individual crops and even specific ages of a specific crop. Presently, the only functional methods available for monitoring plant nutritional status and biochemical quality is destructive sampling of plant material followed by laboratory analysis of the sampled tissue. The current state-of-the-art in monitoring plant health involves a combination of visual examination for observation of symptoms associated with nutritional deficiencies or toxicity and tissue and soil (root media) analysis. The DASI has the potential to provide an assessment of nutrient status, water status, biomass accumulation and biochemical quality, in a non-destructive manner, through analysis of a digital image. It also has the distinct advantage of doing the assessment in real time so that it can be used as a real time management tool for a regenerative life support system.

Presently, the control of nutrient delivery to most hydroponic crop production systems is based on monitoring and maintaining the desired elemental concentrations in the nutrient solution. Direct monitoring of the plant's nutrient level is not used as the control signal because of the difficulty of determining it in real time; thus, indirect maintenance of solution concentrations are used. It is well established that the nutrient requirement differs among various crops and for various developmental stages of a single crop. Long-term operation of a single or small number of hydroponic system supporting a mix of crop types with nutrient and water additions maintained on the current basis of solution electrical conductivity is prone to potential nutrient imbalance. While the primary emphasis of the proposal will be on sensing plant stress, the clear potential of the DASI to provide a direct signal regarding the nutritional quality of the plant material will be considered. This is an especially attractive technique given the potential to view specific components, potentially at the individual plant level, within the overall mixture of the entire crop community on a real time basis.

The goal of the proposal will be to determine the suitability of the DASI instrument in providing a signal that can be recognized and be utilized as an indicator of plant stress. The method to be utilized for evaluating stress is the presentation of an every increasing level of nutrient deficiency and salinity stress (addition of salt $[NaCl]$ or increasing concentration of balanced nutrient) while simultaneously recording spectral reflectance using the DASI instrument and monitoring the traditional processes of gas exchange and nutrient uptake parameters. In this manner, we will be able to directly compare the DASI measurements with known stresses as

determined by the traditional gas exchange and nutrient uptake measures of stress. We anticipate that the DASI will provide a sensitive identifier of plant stress; recording signals of the resulting changes in plant metabolism in real time, far before any visible effects of stress could be observed. Thus, there is a potential for very early management intervention to correct a stress condition before damage could develop.

The present response time for the observation of visual symptoms of plant stress is considerable and only provides an indication that a stress is present after it has been present for an extended period of time. Thus, the impact of a plant-based life support function will have already been significant. An additional benefit of this research to regenerative life support will be the characterization of a potential recovery scenario from various degrees of stress. The experimental approach to be employed includes the removal of the stress at various points in the stress gradient and the characterization of plant performance and reflectance spectra during recovery from various degrees of stress.

Spectral reflectance imaging techniques have been developed and used to measure the biochemical composition of plants and relate these characteristics to the fluxes of biogenic elements within the ecosystem. The technique has been used to directly measure the concentration of plant constituents (nitrogen, protein, lignin, cellulose, chlorophyll, and others), to recognize the onset of water deficiency, and to estimate biomass accumulation and partitioning within the plant. In a range of field evaluations, the results provided with the DASI have been correlated with direct, destructive laboratory analysis of biomass collected from the field. Since this work has primarily involved field and airborne measurements, the sensitivity of the technique and the instrument to measure biochemical composition and environmentally induced stress responses has not been fully characterized. With the quantifiable and repeatable presentation of stress in controlled environments included in the proposal, the sensitivity of the DASI in recognizing changes in important plant components or processes prior to realizing any negative impact on plant-based, life-support processes will be determined and compared with current physiological practices.

It is anticipated that the results derived from this experimental plan will determine the potential for application of spectral reflectance analysis in monitoring and control of crop performance in a regenerative life support system as well as enhance the interpretative capabilities in field applications of the DASI.